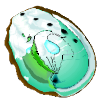
State of California
Environmental Laboratory Accreditation Program



AQUATIC TOXICITY TESTING

(Fields of Testing 113 and 119)

INTRODUCTION

This section covers the general certification requirements for aquatic toxicity testing of effluents and hazardous wastes.

Laboratory aquatic bioassay procedures will be evaluated based on comparison with protocols approved by the State Water Resources Control Board or the Department of Health Services. The approved methods are listed on our Field of Testing (FoT) worksheets that are available from our website (http://www.dhs.ca.gov/ps/ls/elap/elapindex.htm).

CERTIFICATION REQUIREMENTS

Environmental Laboratory Accreditation Program (ELAP) Certification for aquatic toxicity testing requires the following:

1. Successful completion of on-site audit, including satisfactory response to all deficiencies noted.

2. Approval of laboratory Quality Assurance (QA) manual and Standard Operating Procedures (SOPs) appropriate to toxicity tests performed.

3. Acceptable analysis of proficiency testing samples for each subgroup if available.

The FoT worksheets and all SOPs should be submitted to ELAP before the site inspection date. Please submit documents to:

Department of Health Services
Environmental Laboratory Accreditation Program
601 North 7th Street, MS-92
P.O. Box 942732
Sacramento, CA 94234-7320

FACILITIES and EQUIPMENT

Facilities must include:

- Isolated aquatic testing facilities to avoid cross-contamination of tests and stressing test organisms, and for the safety of lab personnel.
- Test containers in sufficient number for sample and reference toxicant tests, using materials required in protocols.
- Holding facilities of appropriate size and type for each species used in testing.
- Sample mixing equipment (appropriate to protocol).
- Disposal facilities for samples, test solutions, and chemicals.
- Air supply (oil-less).
- Adequate illumination system.
- Adequate temperature control.
- Statistical methods for data calculation.

Equipment available to measure:

- Temperature (including a continuously recording thermometer).
- pH (accurate to 0.1 pH unit)
- Dissolved oxygen (accurate to 0.1mg/L)
- Salinity/specific conductance
- Hardness (should be measured according to EPA Method 130.2)
- Alkalinity (should be measured according to EPA Method 310.1)
- Mass (of sufficient sensitivity for type of organism tested)
- Length (to 1 mm for larger organisms)

Special Equipment may be required for:

Static renewal tests:

- Transfer of organisms or renewal of test solutions.

Flow-through tests:

- Continuous flow system constructed of material appropriate to protocols, with enough outflows and containers to run duplicate concentrations.
- Equipment to measure flows.
- Flow-through system for control water.

DATA EVALUATION

Percent survival may be computed manually. For endpoints such as LCs, ECs, ICs, and NOEC/LOEC, computer programs are available and should be utilized. A program may be obtained from ELAP to calculate LC50s using the probit method, the moving average method and the binomial method. Send a request to the address on page one or call (916) 323-4769. Other software available to calculate toxicity endpoints includes Cetis or ToxCalc from Tidepool Scientific Software at (707) 839-5174, http://members.aol.com/tidesoft/cetis/, http://members.aol.com/tidesoft/toxcalc/, and SoftTox from ChemSW at (707) 864-0845, http://www.chemsw.com/.

QUALITY CONTROL

1. **Quality Assurance Plan**

The QA plan developed for the laboratory must be followed and reflect actual laboratory practices. It must include current procedures and be made accessible to all analysts.

2. **Replicates**

Most procedures require at least duplicate test chambers, and the chronic aquatic toxicity tests require multiple replicates.

3. **Controls**

At least one dilution water control must be run with each test. The control must be run under identical conditions to the test chambers. Additional controls will be required during a test where pH or salinity is adjusted, or when a carrier solvent is used.

4. **Food Suitability**

The suitability of the food supply must be tested for EPA chronic tests and should be tested whenever feeding is required during a test.

5. **Reference Toxicants**

It is a laboratory's responsibility to demonstrate its ability to obtain consistent, precise results with reference toxicants before it performs toxicity tests with effluents for permit compliance purposes. To meet this requirement, the intra-laboratory precision of each type of test to be used in a laboratory must be determined by performing five or more tests with different batches of test organisms, using the same reference toxicant, at the same concentrations, with the same test conditions, and the same data analysis methods. In addition, most protocols require reference toxicant testing to evaluate the sensitivity of each batch of test organisms. The type of reference toxicant that should be used is dependent on the type of organism being tested. The table on page 9 lists the recommended toxicants for most species. Selection of a toxicant should be based on safety to analysts, ease of disposal, and ability to generate precise endpoint.

6. **Performance Evaluation Samples**

At the present time, ELAP does not require annual performance evaluation samples for laboratories certified or seeking certification to perform aquatic bioassays. Third-party sample providers are currently being evaluated by NIST and may be approved by the end of this year. ELAP will require successful analyses of these performance evaluation samples when they become available.

TEST ORGANISMS

Test organisms must be killed before disposal. Any surviving organisms, including controls, may <u>not</u> be used in future tests or released.

A permit must be obtained from the Department of Fish and Game if organisms are collected from wild stocks.

A list of registered aquaculturists is available from the Department of Fish and Game Regional offices.

Pages 5 through 8 provide information on suppliers of commonly used aquatic toxicity test organisms. ELAP does not endorse any particular supplier nor is this list inclusive.

FRESHWATER FISH SUPPLIERS

ORGANISM	SUPPLIER	LOCATION	PHONE
Juvenile Fathead Minnows (1-90 day old)	Thomas Fish Company	Anderson, CA	(530) 378-1006
	Environmental Consulting & Testing	Superior, WI	(800) 377-3657
	Sticklebacks Unlimited	Vallejo, CA	(707) 644-6997
	AquaTech Env. Services	Virginia Beach, VA	(800) 899-3638
	Aquatic Biosystems	Fort Collins, CO	(800) 331-5916
	Cosper Environmental	Bohemia, NY	(800) 428-7733
	Aquatox	Hot Springs, AR	(501) 767-9120
Juvenile Sticklebacks	Brezina and Associates	Dillon Beach, CA	(707) 878-2853
	Don Miguel	Bodega, CA	(707) 874-1452
	Sticklebacks Unlimited	Vallejo, CA	(707) 644-6997
Larval and Juvenile Rainbow Trout	Block Environmental	Pleasant Hill, CA	(925) 682-7200
	Lost River Trout Hatchery	Mackay, ID	(208) 588-2866
	Spring Creek Hatchery	Lewiston, MT	(406) 538-3538
	Sticklebacks Unlimited	Vallejo, CA	(707) 644-6997
	Thomas Fish Co.	Anderson, CA	(530) 378-1006
Fathead Minnow Larvae (1-14 day old)	Aquatic Biosystems	Fort Collins, CO	(800) 331-5916
	AquaTech Env. Services	Virginia Beach, VA	(800) 899-3638
	Aquatox	Hot Springs, AR	(501) 767-9120
	Cosper Environmental	Bohemia, NY	(800) 428-7733
	Environmental Consulting & Testing	Superior, WI	(800) 377-3657
	Enviro Sciences	Carrollton, TX	(214) 241-8952
	Sach System Aquacult.	Florida	(904) 824-6308

FRESHWATER TEST ORGANISM SUPPLIERS

ORGANISM	SUPPLIER	LOCATION	PHONE
Water Fleas	Aquatic Biosystems	Fort Collins, CO	(800) 331-5916
	Aquastar	Mobile, AL	(800) 831-9279
	Cosper Environmental	Bohemia, NY	(800) 428-7733
	Environmental Consulting & Testing	Superior, WI	(800) 377-3657
	Enviro Sciences	Carrollton, TX	(214) 241-8952
Green Algae	Aquatic Biosystems	Fort Collins, CO	(800) 331-5916
	American Type Culture Collection (Culture No. ATCC 22662)	Manassas, VA	(703) 365-2700
	Aquatic Biology Branch, Quality Assurance Research Division, EPA	EMSL-Cincinnati, Newtown Facility	(513) 533-8114
	Environmental Consulting & Testing	Superior, WI	(800) 377-3657
	Enviro Sciences	Carrollton, TX	(214) 241-8952
	Culture Collection of Algae, Botany Dept., University of Texas	Austin, TX	(512) 471-4019
	Environmental Research Laboratory, USEPA	Corvallis, OR	(503) 754-4600

MARINE TEST ORGANISM SUPPLIERS

ORGANISM	SUPPLIER	LOCATION	PHONE
Abalone	Abalone Farm	Cayucos, CA	(805) 995-2495
	Ablab	Port Hueneme, CA	(805) 488-6137
	Abalone International	Crescent City, CA	(707) 464-6913
	The Cultured Abalone	Santa Barbara, CA	(805) 685-1956
	Kim Siewers	Santa Cruz, CA	(408) 425-1391
	McCormick and Associates	Ojai, CA	(805) 488-1041
	Pacific Biomarine	Inglewood, CA	(310) 822-5757
Bivalves	Brezina and Associates	Dillon Beach, CA	(707) 878-2853
	Carlsbad Aquafarm	Carlsbad, CA	(760) 438-2444
	Dave Gutoff	San Diego, CA	(619) 685-7647
	Kim Siewers	Santa Cruz, CA	(408) 425-1391
Red Algae and Diatoms	Botany Department, University of Texas	Austin, TX	(512) 471-4019
Giant Kelp sporophylls	Canestro and Associates	Santa Barbara, CA	(805) 893-2476
	Dave Gutoff	San Diego, CA	(619) 685-7647
	Kim Siewers	Santa Cruz, CA	(408) 425-1391
	McCormick and Associates	Ojai, CA	(805) 488-1041
	Pacific Biomarine	Inglewood, CA	(310) 822-5757

MARINE TEST ORGANISM SUPPLIERS

ORGANISM	SUPPLIER	LOCATION	PHONE
Silversides and Topsmelt	Aquatic Biosystems	Fort Collins, CO	(800) 331-5196
	Aquatic Indicators	St. Augustine, FL	(904) 829-2780
	Aquatox	Hot Springs, AR	(501) 767-9120
	Linnt Trout Farm	Half Moon Bay, CA	(415) 726-0845
	Aquatic Resources	Sebastapol, CA	(707) 829-3829
Holmesimysis costata	Dave Gutoff	San Diego, CA	(619) 685-7647
	Kim Siewers	San Cruz, CA	(408) 425-1391
	Pacific Biomarine	Inglewood, CA	(310) 822-5757
Mysidopsis bahia	Aquatic Biosystems	Fort Collins, CO	(800) 331-5196
	Aquatic Indicators	St. Augustine, FL	(904) 829-2780
	Aquatox	Hot Springs, AR	(501) 767-9120
Neomysis mercedis	Brezina and Associates	Dillon Beach, CA	(707) 878-2853
Sand Dollars and Sea Urchins	Dave Gutoff	San Diego, CA	(619) 685-7647
	Kim Siewers	Santa Cruz, CA	(408) 425-1391
	Pacific Biomarine	Inglewood, CA	(310) 822-5757
	Seacology	Vancouver, BC	(604) 737-2106
	Marinus, Inc.	Long Beach, CA	(562) 435-6522
Sanddabs	Aquatic Biosystems	Fort Collins, CO	(800) 331-5196
	Brezina and Associates	Dillon Beach, CA	(707) 878-2853
	Enviro Sciences	Carrolton, TX	(214) 241-8952
	Pacific Biomarine	Inglewood, CA	(310) 822-5757

RECOMMENDED REFERENCE TOXICANTS

SPECIES	REFERENCE TOXICANT	PROTOCOL
Red alga Champia parvula	Sodium dodecyl sulfate (SDS), cadmium chloride, copper sulfate	EPA/600/4-87/028
Alga Selenastrum capricornutum	SDS, copper sulfate, sodium chloride, cadium chloride, etc.	EPA/600/4-89/001
Shrimp Mysidopsis bahia Holmesimysis costata	SDS, cadmium chloride, copper sulfate zinc sulfate	EPA/600/4-87/028 EPA/600/R-95/136
Water flea Ceriodaphnia dubia	SDS, copper sulfate, sodium chloride, cadmium chloride, etc.	EPA/600/4-89/001
Giant kelp Macrocystis pyrifera	copper chloride	EPA/600/R-95/136
Red abalone Haliotis rufescens	zinc sulfate	EPA/600/R-95/136
Oyster Crassostrea gigas	copper sulfate, sodium azide, copper chloride	ASTM E724-87 EPA/600/R-95/136
Mussel Mytilis edulis	copper sulfate, sodium azide, copper chloride	ASTM E724-87 EPA/600/R-95/136
Echinoderms Strongylocentrotus purpuratus Strongylocentrotus franciscanus Dendraster excentricus	silver nitrate, SDS, copper sulfate copper chloride	Dinnel, 1987 EPA/600/R-95/136
Silversides Menidia beryllina	SDS, cadmium chloride, copper sulfate	EPA/600/4-87/028
Fathead minnows Pimephales promelas	SDS, copper sulfate, sodium chloride, cadmium chloride, etc.	EPA/600/4-89/001